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Improved procedure for the purification of selenoprotein Ph from human plasma.

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Selenoprotein Ph, the human analogue of selenoprotein P from rat plasma, was purified from human plasma using Heparin Sepharose chromatography, PEG precipitation, DEAE ion exchange chromatography. RP chromatography, SDS-PAGE and electroelution. SDS-PAGE of the purified protein revealed one broad-selenium containing protein band from 56 to 67 kDa with a selenium maximum at 62 kDa. Using a 7.5% T gel this band was separated into two distinct selenium-containing bands with molecular weights of 61 and 64 kDa.

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Purification of selenoprotein Ph from human plasma.

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There are three selenium-containing proteins in human plasma: glutathione peroxidase (GSH-Px-P), albumin and selenoprotein Ph, the human analogue to selenoprotein P from rat plasma. Selenoprotein Ph was separated from the two other selenium-containing proteins by Heparin Sepharose chromatography and was shown to have about 60-70% of the total plasma selenium, while both GSH-Px-P and albumin contain about 15%. A 2588-fold purification from human plasma was achieved by using a four-step procedure. SDS-PAGE of the purified selenoprotein revealed, besides one contaminant selenium-free protein band at about 70 kDa, one selenium-containing band ranging from 54 to 67 kDa with a maximum at 63 kDa. This microheterogeneity, also recognized by IEF, may be due to the glycprotein nature of the selenoprotein Ph. The determination of the molecular mass of the native protein varied from 65 kDa using gel filtration on Fraktogel HW 55 to 89 kDa on Sephacryl S-200 HR, suggesting an interaction between the gel-matrices and selenoprotein Ph.

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